

FORM PTO-1300 (Modified) (REV 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER <b>225-01</b>	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)	
				<b>09/936755</b>	
INTERNATIONAL APPLICATION NO. <b>PCT/JP00/04422</b>		INTERNATIONAL FILING DATE <b>July 4, 2000</b>		PRIORITY DATE CLAIMED <b>August 27, 1999</b>	
TITLE OF INVENTION <b>ACIDIC PROTEIN FOODS, PROCESS FOR THEIR PRODUCTION AND STABILIZER</b>					
APPLICANT(S) FOR DO/EO/US <b>Taro Takahashi, et al.</b>					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.</li> <li>4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).             <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is attached hereto.</li> <li>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</li> </ol> </li> <li>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input type="checkbox"/> have been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4))</li> <li>10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).</li> <li>11. <input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409).</li> <li>12. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210).</li> </ol>					
<b>Items 13 to 20 below concern document(s) or information included:</b>					
<ol style="list-style-type: none"> <li>13. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>14. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>15. <input type="checkbox"/> A <b>FIRST</b> preliminary amendment</li> <li>16. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment</li> <li>17. <input type="checkbox"/> A substitute specification.</li> <li>18. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>19. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.</li> <li>20. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</li> <li>21. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</li> <li>22. <input checked="" type="checkbox"/> Certificate of Mailing by Express Mail</li> <li>23. <input type="checkbox"/> Other items or information</li> </ol>					

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)

INTERNATIONAL APPLICATION NO.

ATTORNEY'S DOCKET NUMBER

09/936755

PCT/JP00/04422

225-01

24. The following fees are submitted.

**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :**

**CALCULATIONS PTO USE ONLY**

- ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... \$1000.00
- ☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... \$860.00
- ☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$710.00
- ☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$690.00
- ☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$100.00

**ENTER APPROPRIATE BASIC FEE AMOUNT =**

\$860.00

Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)).

☐ 20 ☐ 30

\$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	7 - 20 =	0	x \$18.00	\$0.00
Independent claims	4 - 3 =	1	x \$80.00	\$80.00
Multiple Dependent Claims (check if applicable)			<input type="checkbox"/>	\$0.00

**TOTAL OF ABOVE CALCULATIONS = \$940.00**

- ☐ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.

\$0.00

**SUBTOTAL = \$940.00**

Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)).

☐ 20 ☐ 30

\$0.00

**TOTAL NATIONAL FEE = \$940.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).

☒

\$40.00

**TOTAL FEES ENCLOSED = \$980.00**

Amount to be:  
refunded \$  
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- a. ☒ A check in the amount of \$940.00 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. in the amount of to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 16-0750. A duplicate copy of this sheet is enclosed.
- d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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NAME

23,028

REGISTRATION NUMBER

September 17, 2001

DATE

## DESCRIPTION

ACIDIC PROTEIN FOODS, PROCESS FOR THEIR PRODUCTION AND  
STABILIZER

5

Technical Field

The present invention relates to acidic protein foods, to a process for their production and to a stabilizer for acidic protein foods.

10

Background Art

Proteins in acidic milk beverages such as "liquid yogurt", lactic acid bacteria beverages, fruit milk and the like are highly unstable at near their isoelectric point of pH 3.8-5.3, such that the milk proteins coagulate, and with passage of time the milk proteins precipitate resulting in separation of the whey. Upon sterilization heating, this coagulation becomes severe to a point where the product value is totally lost.

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In the past, it has been common to use pectin at 0.2-0.4 wt% in such acidic milk beverages such as liquid yogurt and lactic acid bacteria beverages in order to achieve stable dispersion of the proteins.

25

Pectin is an acidic polysaccharide typically present in high quantities in citrus fruits, apples, quince and the like, and galacturonic acid in the molecules can form crosslinking between proteins, resulting in stable dispersion of the proteins in the acidic milk beverages for given periods of time.

30

However, while addition of pectin can improve the stability of acidic milk beverages, it also increases their viscosity at the same time, resulting in a pasty feel, impairing the swallowability, or in extreme cases causing gelling, such that the manageability and product value are reduced.

35

There have been reported attempts to add low molecular pectin, subjected to enzymolysis or the like,

to acidic milk beverages in order to reduce the viscosity of the acidic milk beverages (Carbohydrate Polymers, 6, P.361-378(1986), Japanese Unexamined Patent Publication No. 7-264977), but in all such cases it has been  
5 indicated that the stability is lowered with reduced molecular weight and that the use of low-molecularized pectin alone cannot stabilize acidic milk beverages.

Other inventions relating to the use of low-molecularized pectin include an invention employing  
10 thermolytically low-molecularized pectin as an emulsifier (which exhibits surface activity in water-in-oil systems) or emulsion stabilizer (which stabilizes water-in-oil emulsions) (Japanese Unexamined Patent Publication No. 10-4894), and an invention employing low-molecularized  
15 pectin obtained by action of endo-polygalacturonase on pectin, as food fiber (Japanese Unexamined Patent Publication No. 5-252972); however, none of these inventions teach that low-molecularized pectin obtained by pectin degradation contributes to dispersion stability  
20 of milk proteins under acidic conditions.

Moreover, the maximum pH of acidic milk beverages that can be stabilized by conventional pectin is at highest the isoelectric point of the proteins used, or up to pH 4.5 in the case of milk proteins, and since there  
25 has been no technique for stabilization of acidic milk beverages without production of a pasty feel in a pH range which is higher than the isoelectric point, there has been a limit to the flavors of acidic milk beverages that can be used.

30

#### Disclosure of the Invention

It is an object of the present invention to provide acidic protein foods that simultaneously achieve improved  
35 stability of proteins under acidic conditions and enhanced palatable texture due to lower viscosity of the products, as well as a process for their production and to a stabilizer for acidic protein foods.

As a result of much diligent research directed toward achieving the object stated above, the present inventors have found that incorporation of low-molecularized pectin in acidic protein foods at above a specified content can stabilize the dispersion of milk proteins in the acidic protein foods while also lowering the viscosity of the acidic protein foods to give the products a more palatable texture, and can also widen the pH range in which stabilization is possible to above the isoelectric point. The present invention has been completed on the basis of this finding.

The invention therefore provides acidic protein foods containing low-molecularized pectin at greater than 0.4 wt%. The pectin is preferably low-molecularized to a degree such that the viscosity of a 5% solution at 25°C is no greater than 150 mPa·s, and the acidic protein foods are typically beverages. The viscosity of the obtained beverages may be up to 10 mPa·s at 25°C.

The invention further provides a process for production of acidic protein foods which comprises using low-molecularized pectin at greater than 0.4 wt%, as well as a process for production of acidic protein foods whereby acidic protein foods containing non-low-molecularized pectin at greater than 0.4 wt% are heated at 100°C or above. The invention still further provides a stabilizer for acidic protein foods which contains low-molecularized pectin as an active ingredient.

#### Best Mode for Carrying Out the Invention

The low-molecularized pectin used for the invention may be obtained by any known chemical or physical treatment capable of lowering the degree of polymerization of the pectin. As examples of such treatment there may be mentioned acid hydrolysis treatment, ultrasonic treatment, gamma ray treatment, mechanical decomposition treatment by shear force,  $\beta$ -elimination treatment with an alkali, oxidative

decomposition using an oxidizing agent such as oxygen, chlorine, chlorite, hypochlorite, hydrogen peroxide, Fenton's reagent or the like, or decomposition with enzymes or microorganisms. When using an enzyme, pectin lyase, polygalacturonase, and combinations of pectin esterase and polygalacturonase are suitable.

The starting material for the low-molecularized pectin of the invention may be any source such as citrus fruits, apples, quince, roots or the like. Pectin obtained from citrus fruits, apples and quince, and particularly a high methoxyl type with a degree of esterification (DE value) of 50% or greater, is preferred from the standpoint of exhibiting an effect on dispersion stability across a wide range of low-molecularization.

Thermolytic treatment is preferred for preparation of the low-molecularized pectin, and this will be explained in detail below. Pectin is usually obtained by suspension of the raw source, i.e. citrus fruit peel or apple juice sediment in water, adjustment of the pH to 1-6, and then extraction under mechanical shear at a temperature of no higher than 100°C and preferably 75-85°C, for a period of from a few minutes to a few hours, and preferably 30-120 minutes. The pectin extracted in this manner is then separated and collected from the suspension by centrifugal precipitation removal and/or by filtration using an aid such as perlite, diatomaceous earth or cellulose. The collected pectin solution is then subjected to thermolytic treatment involving heating to above 100°C, preferably 100-130°C, and maintenance of that temperature for a few minutes, preferably 5-15 minutes, to prepare low-molecularized pectin. The resulting low-molecularized pectin may be adequately concentrated by ultrafiltration or evaporation before precipitation with an alcohol (or metal salt), or before direct drying. This low-molecularized pectin obtained by thermolysis is then dried and powdered.

In this process, the low-molecularization and

extraction of the pectin may be accomplished simultaneously, in which case the extraction of the pectin must be carried out in a temperature range in which the pectin undergoes decomposition, i.e. at 100°C or above (under normal pressurized conditions), and preferably 100-130°C. The extraction time is set so that the viscosity of the pectin is within the specified range, and it may be determined as appropriate depending on the treatment temperature, after confirmation by preliminary experimentation.

The low-molecularization of the pectin may be accomplished at another stage of the industrial process, such as directly, or else during any of the steps of concentration, precipitation, powdering, etc. which are carried out after redissolution or reprecipitation under solventless conditions.

For enzymolysis, an enzyme with pectin-degrading activity may be added to a 1-5% pectin aqueous solution, and stirring carried out at 15-55°C for 2 minutes to 24 hours followed by heating for inactivation of the enzyme. The low-molecularized pectin of the invention may remain in the form of the aqueous solution, but alternatively it may be powdered by addition of an alcohol to the treatment solution to precipitate the low-molecularized pectin, or spray drying.

The pectinase used for preparation of the low-molecularized pectin of the invention is not particularly limited so long as it can decompose and reduce the molecular weight of the pectin, and as examples there may be mentioned pectin lyase, polygalacturonase, and combinations of pectin esterase and polygalacturonase.

The low-molecularized pectin of the invention is decomposed to an extent at which it exhibits a viscosity of 150 mPa·s or lower when measuring a 5% aqueous solution with a type-B viscometer (Rotor No. 1, 60 rpm, 25°C).

On the other hand, the low-molecularized pectin of

the invention preferably exhibits a viscosity of 13 mPa·s or greater, more preferably 15-130 mPa·s and even more preferably 20-90 mPa·s for a 5% aqueous solution. The viscosity of 5% aqueous solutions of non-decomposed pectin is commonly 200 mPa·s or greater, and even more commonly 400 mPa·s or greater, and these can be altered depending on the source of the pectin and by the chemical properties such as the DE value.

When low-molecularized pectin according to the invention is used to produce acidic protein foods, it may be added in an amount exceeding 0.4 wt% with respect to the acidic protein foods, which has been considered unsuitable for conventional non-decomposed pectin. When low-molecularized pectin is used, it is not possible to achieve adequate dispersion stability at lower than 0.4 wt%. Addition in an amount of preferably 0.5 wt% or greater and more preferably 0.7 wt% or greater with respect to the acidic milk beverages can widen the pH range in which stabilization is possible to above the isoelectric point of the proteins.

While there are no particular restrictions on the upper limit for addition, it is preferably used at no greater than 5.0 wt% in the acidic protein foods in order to limit the viscosity of acidic milk beverage products to 10 mPa·s to prevent a pasty feel or impaired swallowability.

Reduction in the product viscosity for production of an acidic protein food according to the invention can be achieved by adding non-decomposed or low-molecularized pectin to an acidic milk beverage in an amount exceeding 0.4 wt%, preferably 0.5 wt% or greater and more preferably 0.7 wt% or greater, for stabilization of the acidic protein food, and then heating and especially retort heating thereof to 100°C or higher. Conventional acidic milk beverages require no sterilization treatment at 100°C or above because of their acidic range, and since addition of large amounts of pectin raises the



viscosity, pectin has not been added to acidic milk beverages in amounts exceeding 0.4 wt%.

The acidic protein foods of the invention are acidic protein foods containing animal and vegetable proteins, and they include acidic protein beverages prepared by adding citrus juices or other juices, or organic acids such as citric acid and lactic acid, or inorganic acids such as phosphoric acid, to beverages comprising animal or vegetable proteins such as milk, soybean milk and the like; acidic milk beverages obtained by acidifying dairy products; acidic frozen desserts such as acidic ice cream and frozen yogurt obtained by adding fruit juices to frozen desserts containing milk components, such as ice cream and the like; acidic desserts obtained by adding fruit juices, etc. to gelled foods such as puddings and Bavarian cream, as well as acidic milk beverages such as lactic acid bacteria beverages (including live bacteria and sterilized types), fermented milk (in solid or liquid form), and the like. Animal and vegetable proteins include cow milk, goat milk, skim milk, soybean milk and whole milk powder or skim milk powder obtained by powdering these, as well as sugar-added milk, condensed milk, processed milk obtained by fortification with minerals such as calcium or vitamins, fermented milk, and the proteins obtained from the same. Fermented milk refers to fermented milk obtained by addition of a lactic acid bacteria starter and fermentation after sterilization of the animal or vegetable proteins, and it may be further powdered or sugar may be added thereto, as desired.

Examples are provided below for a more detailed explanation of the preferred mode of the invention, with the understanding that they are exemplary and are not intended to restrict the spirit of the invention. The "parts" and "%" values in the examples are based on weight.

Example 1

a) Preparation of low-molecularized pectin by enzymolysis

After decomposition and dissolution of 100 g of pectin with a DE value of 71% (trade name: "Pectin SM-478", by San-Ei Gen FFI Co., Ltd.) in approximately 1800 g of desalted water heated to 85°C, the solution was cooled to 30°C and desalted water was added to a total volume of 2000 g, and then 1.0 g of a pectin lyase enzyme solution (trade name: "Pektolase LM", by Grinstead Products Co.) was added, and stirring was continued for 2 hours for decomposition reaction. After 2 hours had passed, the treatment solution was contacted with a boiling water bath to deactivate the enzyme, after which the solution was cooled and corrected for the evaporated moisture, and then the insoluble portion was removed by centrifugal separation and the low-molecularized pectin solution was recovered. The recovered sample was placed in a thermostatic water tank at 25°C for 60 minutes, and measurement of the viscosity with a type-B viscometer (Rotor No. 1, 60 rpm, 25°C) gave a viscosity value of 58.0 mPa.s. The obtained low-molecularized pectin was used to prepare acidic milk beverages with the compositions shown in Table 1, and their stability was evaluated. The viscosity of a 5% solution of the non-decomposed pectin before enzymolysis was 279.0 mPa.s.

Table 1

Composition for evaluation of milk protein dispersion  
stabilizing function

Pectin	(5% solution)	6, 10 and 14 parts
Sugar solution	(50% solution)	18 parts
Milk		50 parts
Citric acid solution	Adjusted from a pH of 3.8 to 5.3 with a 50% solution	
Water	Adjusted to a total of 100 parts	

- 5           b) Simple stability test for low-molecularized  
pectin

          An acidic milk beverage was prepared with the  
composition of Table 1 to a total of 1000 g.  
Specifically, a 50% citric acid solution was added  
10 dropwise to milk beverages comprising the pectin solution  
to amounts of 0.3 part (0.3% with respect to the total of  
the acidic milk beverage), 0.5 part (0.5%) and 0.7 part  
(0.7%) of the low-molecularized pectin prepared in a),  
and then the pH of each product was adjusted from 3.8 to  
15 5.3 in increments of 0.3. After pH adjustment, a  
homogenizer was used for homogenization at 150 kgf/cm<sup>2</sup> to  
complete the milk beverages. The viscosity was measured  
with a type-B viscometer (Rotor No. 1, 60 rpm, 25°C) and  
the condition was observed, for each of the obtained  
20 acidic milk beverage products. A 200 g portion of each  
of the acidic milk beverages was filled into a retort  
bag, sterilization was carried out at 80°C for 20 minutes  
or at 121°C for 10 minutes, and the condition was  
observed. The results of evaluation are summarized in  
25 Table 2.

Table 2

Low-molecularized pectin content	Acidic milk beverage preparation pH	Viscosity (mPa-s)	Conditions		
			Before heating	After 80°C sterilization	After 121°C sterilization
0.7 part (0.7%)	pH 3.8	24.8	coagulation	notable coagulation	notable coagulation
"	pH 4.1	7.2	stable	stable	stable
"	pH 4.4	6.5	stable	stable	stable
"	pH 4.7	6.2	stable	stable	stable
"	pH 5.0	9.8	stable	stable	stable
"	pH 5.3	7.4	coagulation	notable coagulation	notable coagulation
0.5 part (0.5%)	pH 3.8	19.5	coagulation	notable coagulation	notable coagulation
"	pH 4.1	5.2	stable	stable	stable
"	pH 4.4	5.6	stable	stable	stable
"	pH 4.7	6.0	stable	stable	stable
"	pH 5.0	9.8	stable	coagulation	notable coagulation
"	pH 5.3	7.4	coagulation	notable coagulation	notable coagulation
0.3 part (0.3%)	pH 3.8	25.7	coagulation	notable coagulation	notable coagulation
"	pH 4.1	8.0	coagulation	coagulation	notable coagulation
"	pH 4.4	5.3	slight coagulation	coagulation	notable coagulation
"	pH 4.7	10.0	coagulation	notable coagulation	notable coagulation
"	pH 5.0	16.1	coagulation	notable coagulation	notable coagulation
"	pH 5.3	5.7	coagulation	notable coagulation	notable coagulation

As clearly seen by the results in Table 2, using the low-molecularized pectin as a stabilizer in an amount of 0.5 part (0.5%) or greater according to the invention improves the stability of acidic milk beverages while also lowering the viscosity. The stabilized products maintained their stable condition even after retort sterilization at 121°C.

#### Example 2

c) Preparation of low-molecularized pectin by thermolysis

After dispersion and dissolution of 100 g of pectin with a DE value of 71% (trade name: "Pectin SM-478", by San-Ei Gen FFI Co., Ltd.) in approximately 1800 g of desalted water heated to 85°C, the solution was cooled to 30°C and desalted water was added to a total volume of 2000 g, and then 100 g portions were filled into retort bags for thermolytic treatment under the conditions shown in Table 3. After the thermolysis, the recovered samples were placed in a thermostatic water tank at 25°C for 60 minutes, and the viscosity was measured with a type-B viscometer (Rotor No. 1, 60 rpm, 25°C). The results are shown in Table 3.

Table 3

No.	Thermolysis conditions	5% solution viscosity
1	Control (unheated)	263.0
2	80°C, 10 min.	262.5
3	100°C, 10 min.	190.0
4	105°C, 10 min.	162.0
5	110°C, 10 min.	126.0
6	115°C, 10 min.	85.5
7	121°C, 10 min.	54.5
8	124°C, 10 min.	33.1
9	128°C, 10 min.	18.7
10	121°C, 20 min.	24.0
11	121°C, 30 min.	12.8

The above results confirmed that thermolysis at 100°C or higher produces a reduction in viscosity of pectin solutions.

Each of the low-molecularized pectin solutions (5%) obtained in c) above were used to prepare acidic milk beverages with the composition shown in Table 4, and their stability was evaluated.

Table 4

Composition for evaluation of milk protein dispersion stabilizing function

Pectin solution	(5% solution)	14 parts
Sugar solution	(50% solution)	18 parts
Milk		50 parts
Citric acid solution	Adjusted to a pH of 4.7 with a 50% solution	
Water	Adjusted to a total of 100 parts	

d) Simple stability test for low-molecularized pectin

An acidic milk beverage was prepared with the composition of Table 4 to a total of 1000 g. Specifically, pectin was added in an amount of 0.7 part each (0.7% with respect to the total of the acidic milk beverage), a 50% citric acid solution was added dropwise, and then the pH was adjusted to 4.7 and a homogenizer was

used for homogenization at 150 kgf/cm<sup>2</sup> to complete the product. The viscosity of the obtained acidic milk beverage product was measured with a type-B viscometer (Rotor No. 1, 60 rpm, 25°C) and the condition thereof was observed. After filling 50 g portions of the acidic milk beverage into retort bags, sterilization was carried out at 80°C for 20 minutes and at 121°C for 10 minutes, after which the condition was observed and the following parameters were measured.

10       Whey separation evaluation:

15       A 20 g portion of the heat sterilized evaluation beverage was precisely measured out and subjected to centrifugal separation for 20 minutes at 750 g using a centrifugal separator (model H-103N, by Kokusan Co., Ltd.), and after observation of the whey separation, the whey was removed and set onto filter paper for 20 minutes, after which the whey was thoroughly removed and the precipitate weight measured, and the milk protein precipitation rate was determined by the following formula.

20       Milk protein precipitation rate (%) = weight of precipitate/weight of sample x 100

25       Table 5 shows the viscosity of each of the acidic milk beverages and the change in the condition of the acidic milk beverages and the milk protein precipitation rates after sterilization.

Table 5

No.	Pectin solution viscosity (5%)	Upper row: Condition (precipitation rate)/ lower row: Viscosity (palatable texture)*		
		Before heating	After 80°C sterilization	After 121°C sterilization
1	263.0	stable (0.8%) 36.1 (++)	stable (0.7%) 36.2 (++)	stable (0.9%) 3.9 (-)
2	262.5	stable (0.5%) 33.5 (++)	stable (0.7%) 33.2 (++)	stable (1.1%) 3.6 (-)
3	190.0	stable (0.6%) 20.5 (++)	stable (0.8%) 18.9 (++)	stable (1.0%) 3.4 (-)
4	162.0	stable (0.7%) 14.3 (++)	stable (0.9%) 14.3 (++)	stable (1.3%) 3.4 (-)
5	126.0	stable (0.5%) 8.9 (-)	stable (0.6%) 8.8 (-)	stable (1.0%) 3.5 (-)
6	85.5	stable (0.5%) 7.2 (-)	stable (0.6%) 7.0 (-)	stable (1.1%) 3.4 (-)
7	54.5	stable (0.8%) 6.9 (-)	stable (0.9%) 7.0 (-)	stable (1.1%) 2.9 (-)
8	33.1	stable (0.8%) 6.2 (-)	stable (1.0%) 6.5 (-)	stable (1.2%) 3.0 (-)
9	18.7	stable (1.2%) 5.8 (-)	stable (1.0%) 6.2 (-)	stable (1.7%) 3.4 (-)
10	24.0	stable (0.8%) 6.0 (-)	stable (1.0%) 6.2 (-)	stable (1.5%) 3.2 (-)
11	12.8	coagulation (10.0%) 13.7 (++)	notable coagulation (23.0%) 4.3 (++)	notable coagulation (14.9%) 3.1 (++)

\*: ++ (pasty feel) ---> - (no pasty feel)

5

As clearly seen by the results in Table 5, by using as a stabilizer pectin which has been low-molecularized to a viscosity of between 13 mPa·s and 130 mPa·s at a 5% solution, it is possible to improve the stability of acidic milk beverages while also lowering the viscosity of the products to no greater than 10 mPa·s, and to enhance the swallowability of the products and avoid a pasty feel.

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Furthermore, the viscosities of the acidic milk beverages after 121°C sterilization were all reduced to under 4.0 mPa·s, no pasty feel was observed, and satisfactory swallowability was exhibited. This confirmed that an equivalent effect is exhibited even with low molecularization of pectin by heating at 100°C or higher after preparation of acidic protein foods.

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### Example 3

Using apple juice sediment (product name: Apple Fiber, by Nitro Co., 5% moisture content) as the pectin

extract starting material, 500 g of the apple juice sediment was suspended in 9500 g of water, the pH was adjusted to 4.5, and then the suspension was heated at 120°C for 30 minutes for simultaneous extraction and low molecularization of the pectin. After cooling, it was subjected to centrifugal separation (10,000g x 30 minutes), the supernatant and precipitate were separated, an equivalent weight of water was added to the separated precipitate, centrifugal separation was repeated, the supernatant was combined with the previous supernatant, after which ethanol was added to an alcohol concentration of 80% for purification of the precipitate. The recovered precipitate portion was lyophilized to prepare low-molecularized pectin. Using this apple juice-derived low-molecularized pectin, the acidic milk beverage stabilizing power at pH 4.7 was observed in the same manner as Example 2, and satisfactory acidic milk with a viscosity of 6.0 mPa·s was obtained. The viscosity of a 5% solution of the low-molecularized pectin was 14.0 mPa·s.

#### Comparative Example 1

Acidic milk beverages with the compositions shown in Table 1 were obtained in the same manner as Example 1 except that pectin with a DE value of 71% (trade name: "Pectin SM-478", by San-Ei Gen FFI Co., Ltd.) was used, and the stability thereof was evaluated. The results of the evaluation are summarized in Table 6.



Table 6

Pectin content	Acidic milk beverage preparation pH	Upper row: Condition (precipitation rate)/ Lower row: Viscosity (palatable texture)*		
		Before heating	After 80°C sterilization	After 121°C sterilization
0.7 part (0.7%)	pH 3.8	stable (1.8%)	little coagulation (1.7%)	coagulation (2.9%)
	"	36.1 (++)	42.2 (++)	12.7 (+)
	pH 4.1	stable (0.5%)	stable (0.8%)	stable (1.3%)
	"	20.5 (++)	15.8 (++)	4.4 (-)
	pH 4.4	stable (0.6%)	stable (0.8%)	stable (1.1%)
	"	29.5 (++)	32.5 (++)	4.3 (-)
"	pH 4.7	stable (0.7%)	stable (0.9%)	stable (1.0%)
	"	36.1 (++)	36.2 (++)	3.9 (-)
	pH 5.0	gelling (1.1%)	sol (1.5%)	stable (1.7%)
"	"	62.2 (++)	42.4 (++)	6.5 (-)
	pH 5.3	coagulation (3.1%)	coagulation (22.5%)	coagulation (18.2%)
	"	50.1 (++)	49.1 (++)	9.3 (+)
0.5 part (0.5%)	pH 3.8	stable (2.4%)	little coagulation (2.5%)	coagulation (18.2%)
	"	19.5 (++)	23.5 (++)	39.3 (++)
	pH 4.1	stable (0.7%)	stable (1.3%)	stable (1.1%)
	"	20.5 (++)	21.8 (++)	3.8 (-)
	pH 4.4	stable (0.6%)	stable (0.8%)	stable (1.0%)
	"	30.2 (++)	29.9 (++)	4.0 (-)
"	pH 4.7	stable (0.7%)	stable (0.9%)	stable (1.3%)
	"	33.5 (++)	35.8 (++)	4.7 (-)
	pH 5.0	stable (2.4%)	little coagulation (2.4%)	coagulation (8.2%)
"	"	49.5 (++)	52.5 (++)	19.3 (++)
	pH 5.3	coagulation (5.4%)	coagulation (22.5%)	coagulation (18.2%)
	"	26.4 (++)	22.5 (++)	7.3 (+)
0.3 part (0.3%)	pH 3.8	coagulation (7.0%)	coagulation (22.5%)	coagulation (16.9%)
	"	22.6 (++)	23.2 (++)	19.3 (++)
	pH 4.1	stable (2.9%)	coagulation (22.5%)	coagulation (20.9%)
	"	16.7 (++)	14.5 (++)	5.6 (-)
	pH 4.4	stable (1.2%)	stable (1.1%)	coagulation (11.5%)
	"	13.5 (++)	18.9 (++)	3.4 (-)
"	pH 4.7	coagulation (12.7%)	coagulation (14.7%)	coagulation (12.5%)
	"	26.4 (++)	22.5 (++)	7.3 (+)
	pH 5.0	coagulation (7.2%)	coagulation (15.9%)	coagulation (9.1%)
"	"	19.5 (++)	23.5 (++)	3.6 (-)
	pH 5.3	coagulation (6.3%)	coagulation (12.4%)	coagulation (15.1%)
	"	19.5 (++)	23.5 (++)	3.6 (-)

\*: ++ (pasty feel) --- - (no pasty feel)

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As clearly shown by the results in Table 6, an increasing amount of pectin addition improves the acidic milk beverage stability, but an increase in viscosity is also observed, while gelling was confirmed with further increase in addition. However, it was confirmed that by using pectin as a stabilizer at 0.5 part (0.5% with respect to the total of the acidic milk beverage) or greater, the stabilized product maintained a stable condition by retort sterilization at 121°C, thus making it possible to achieve lower viscosity for the product.

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#### Industrial Applicability

According to the present invention, acidic milk beverages employing low-molecularized pectin in an amount of greater than 0.4 wt% as acidic milk beverage stabilized compositions can widen the pH range in which stabilization is possible while also achieving low

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viscosity, and therefore improvement in palatable texture, such as swallowability, may be realized.

CLAIMS

1. An acidic protein food containing low-molecularized pectin at greater than 0.4 wt%.
- 5 2. An acidic protein food according to claim 1, wherein the pectin is low-molecularized to a degree such that the viscosity of a 5% solution at 25°C is no greater than 150 mPa·s.
3. An acidic protein food according to claim 1, wherein the acidic protein food is a beverage.
- 10 4. An acidic protein food according to claim 3, wherein the viscosity of the beverage is no greater than 10 mPa·s at 25°C.
5. A process for production of an acidic protein food which comprises adding low-molecularized pectin at greater than 0.4 wt% to an acidic protein food.
- 15 6. A process for production of an acidic protein food whereby the acidic protein food containing non-low-molecularized pectin at greater than 0.4 wt% is heated at 100°C or above.
- 20 7. A stabilizer for an acidic protein food which contains low-molecularized pectin as an active ingredient.

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### Prior Foreign Application(s)

外国での先行出願

11-240787 (Pat. Appln.)

Japan

(Number)

(Country)

(番号)

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I hereby claim foreign priority under Title 35, United States Code, Section 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

### Priority Not Claimed

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27/August/1999

(Day/Month/Year Filed)

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## Japanese Language Declaration

## 日本語宣言書

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ACIDIC PROTEIN FOODS, PROCESS FOR  
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